الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
Garden of Knowledge and Virtue

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on Bioscience and Drug Discovery
"Frontiers in Drug Discovery & Development"
ISBDD 2019
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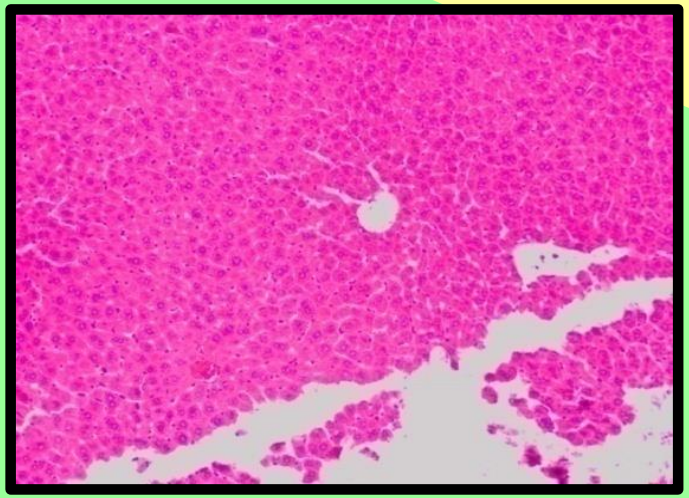
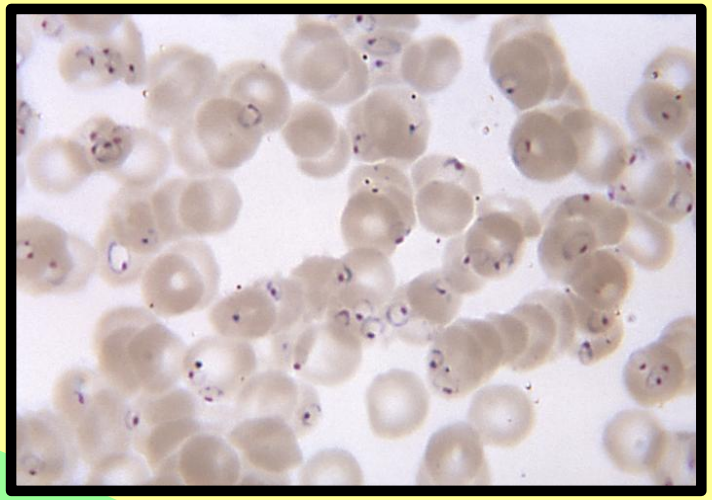
Piper sarmentosum Leaf As a Promising Non-toxic Antimalarial Agent Against *Plasmodium berghei* NK65-Induced Mice





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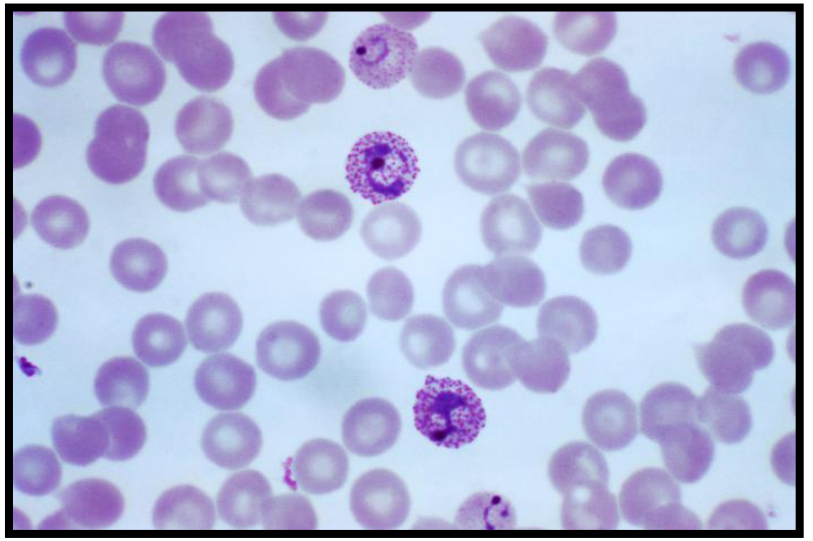
INTRODUCTION

Malaria



- Most threatening and devastating human parasitic vector-borne disease
- *Plasmodium falciparum* → resistance nearly to all current antimalarial drugs
- Side effects of anti-malarial drugs → sulfadoxin, chloroquine, quinine, and pyremethamine
- Increasing in number of malaria cases due to unavoidable factors:

  (a) Wide spread of vector & parasite

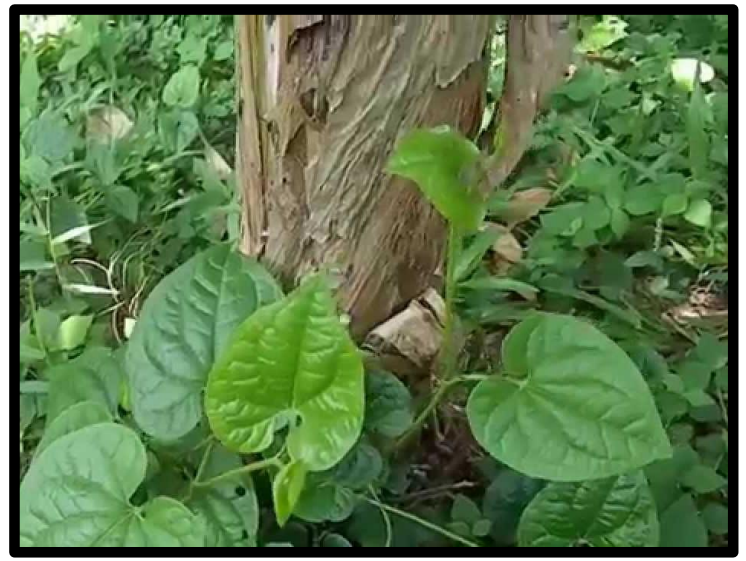
  (b) *P. knowlesi* → new emerging spp.

  (c) Uncontrolled immigrant activity.

  (d) Antimalarial drug resistance strain

Piper sarmentosum

- A traditional herb & aromatic flowering plant locally known as ‘pokok kaduk’ in Malay



- Wildly & abundant in damp open areas, cleared riverbanks and under shady trees (Seyyedan *et al.*, 2013)

- Well growth on cultivated land in India, Sri Lanka & Southeast Asian region (Hussain *et al*., 2009).

- Variety of phytochemical constituents & groups identified from various parts of the plants → phenylpropanoids, α-asarone, asaricin, myricetin, sarmentamide A & B, piperitone, naringenin, spathulenol, farnesol, quercetin, etc…

Piper sarmentosum: The Testimonial

Significant antimicrobial activities (Chan & Wong, 2014):
- Antifungal
- Antiamoebic
- Antituberculosis
- Anti-dengue

Anti-apicomplexial parasite and anti-leishmanial activities of Sarmentamide A (Souza, 2018)

The flowers used to treat many chronic diseases (Shim & Gam, 2012):
- Hypertension
- Diabetes mellitus
- Asthma
- Atherosclerosis

Wide range of pharmacological properties (Syed Ab Rahman *et al*, 2014):
- Wound healing
- Antioxidant
- Anti-infammatory
- Anti-osteoperosis

Antibacterial activities (Sanusi *et al*, 2017): *E. coli, MRSA, B. cereus, V. cholera & S. typhi*

Herbal remedies for many illnesses (Atiax *et al,* 2011):
- Feet dermatitis
- Toothache
- Headaches
- Coughs

Rationale Of The Study

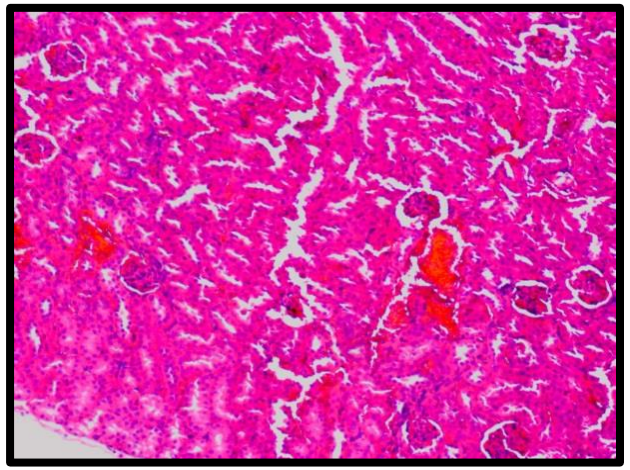
- **Current Antimalarial Drugs**
  - Unaffordable → expensive in certain regions
  - Wrong dosage & concentration → side effects
  - Plasmodial resistance towards antimalarial drugs

- **New Directions On Natural Products Research**
  - Biotechnology → main focus in the next decade
  - Malaysian unexplored forest & natural resources

- ***Piper sarmentosum* (‘pokok kaduk’)**
  - Consumable & easily manipulated
  - Promising documentation on biological testimonies

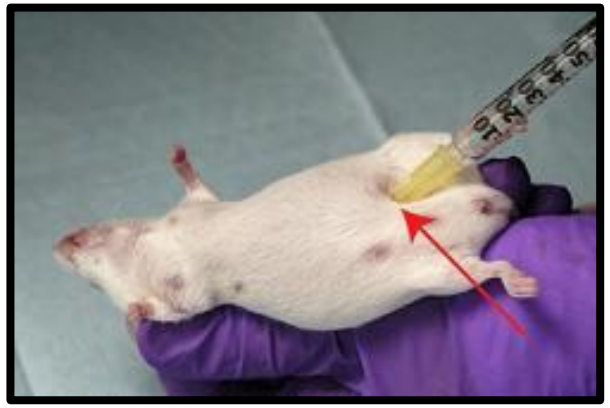
Objectives

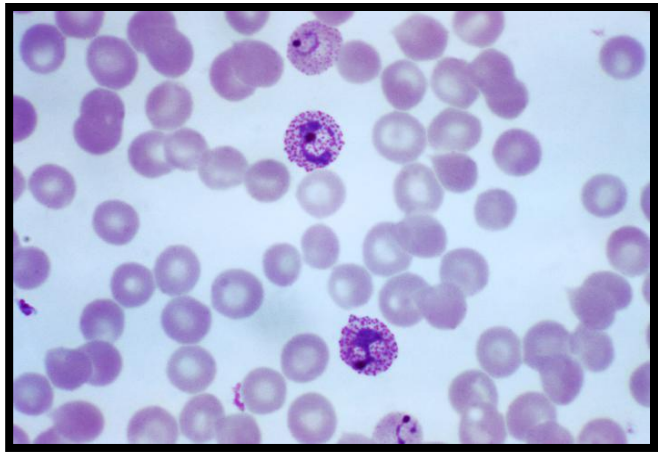
- To evaluate *in-vivo* antimalarial activities of *Piper sarmentosum* leaf aqueous extract against *Plasmodium berghei* N65



- To determine the best regime antimalarial treatment of *Piper sarmentosum* leaf aqueous extract.

- To evaluate the toxicity effects of *Piper sarmentosum* leaf aqueous extract on the infected host

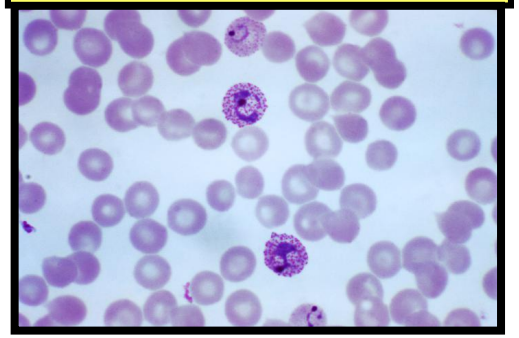




MATERIALS & METHODS

Flow Chart

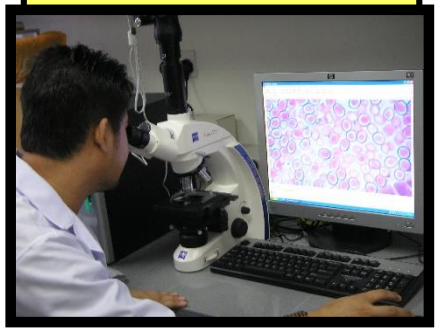
P. berghei **NK65 stock in -80 °C**



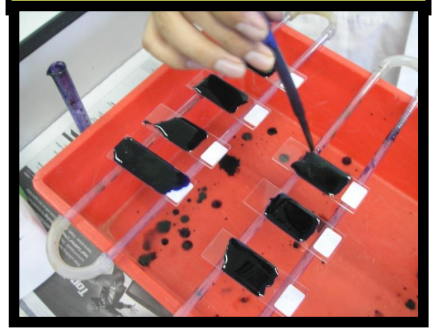
P. berghei **administered i.p. (5 × 10^3** ***T. evansi*****/mice)**

Orally administered of 0.1 mL 10 mg/kg bw of freeze-dried ***P. sarmentosum*** **aqueous extract**

Microscopic observation for inhibition rate evaluation



Giemsa blood slide for parasitemia density



Physical observation of symptoms and mice survival

Blood biochemistry and renal function tests

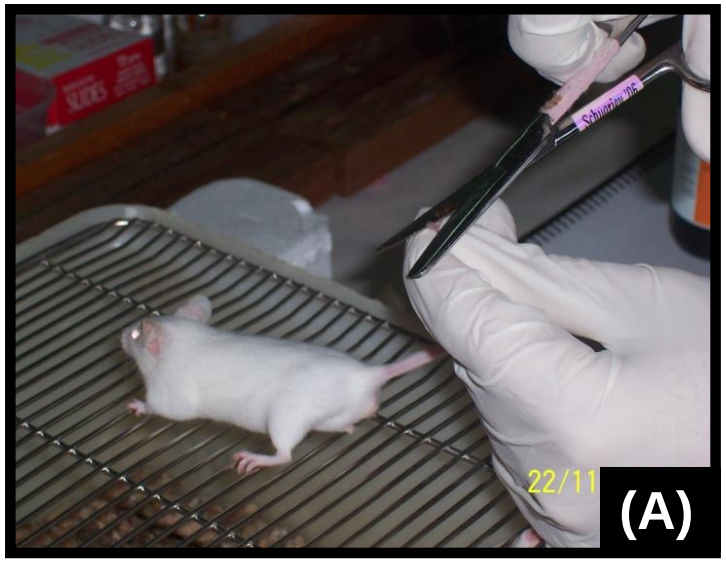
Vital organ histology for toxicity assessment

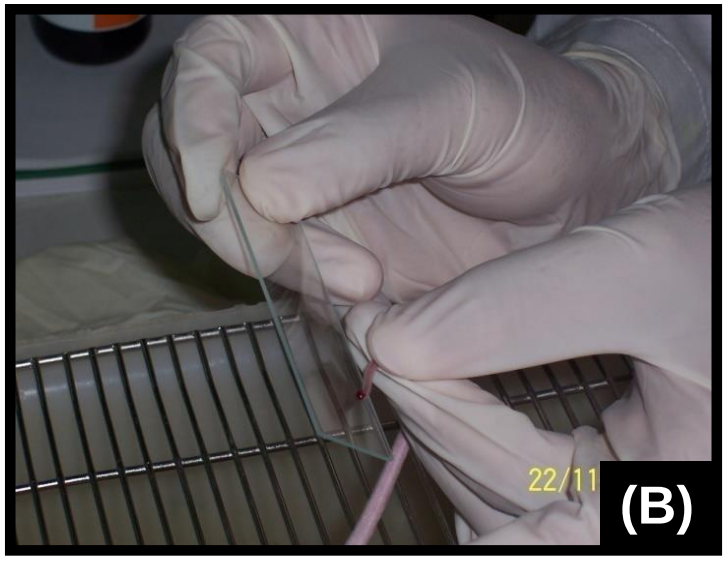
Experimental Design

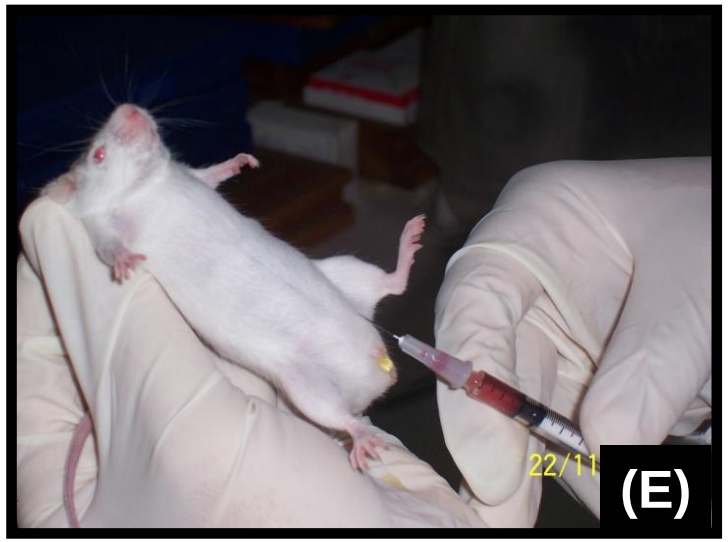
GROUP	REGIMENS	CODE	DESCRIPTION	*P. sarmentosum* DOSAGE
TREATMENT	PREVENTIVE	PRE14	14 days pre-infection	0.2 mL 100 mg/kg bw sdH_2O-extract
		PRE7	7 days pre-infection	0.2 mL 100 mg/kg bw sdH_2O-extract
		PRE3	3 days pre-infection	0.2 mL 100 mg/kg bw sdH_2O-extract
	CURATIVE	CUR3	3 days post-infection	0.2 mL 100 mg/kg bw sdH_2O-extract
		CUR5	5 days post-infection	0.2 mL 100 mg/kg bw sdH_2O-extract
		CUR7	7 days post-infection	0.2 mL 100 mg/kg bw sdH_2O-extract

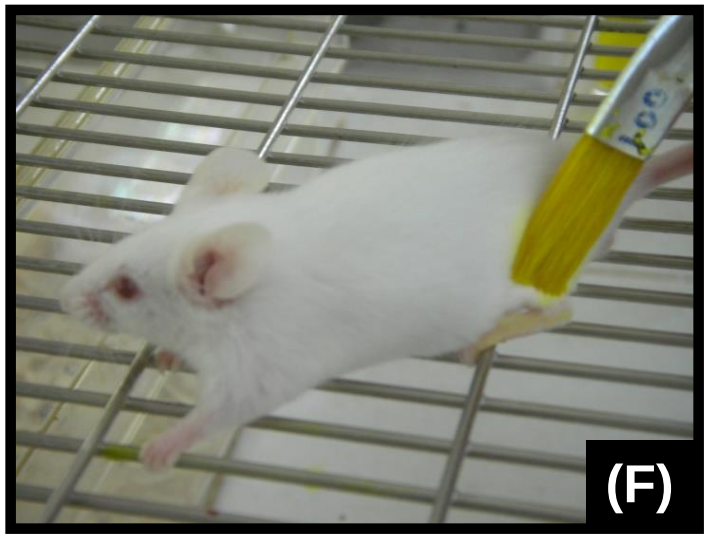
GROUP	REGIMENS	CODE	DESCRIPTION	CONTROL DOSAGE
CONTROL	POSITIVE	PoPRE	Primaquine (daily to D0)	0.1 mL 15 mg/kg bw Primaquine
		PoCUR	Chloroquine (daily from D0)	0.1 mL 10 mg/kg bw Chloroquine
	NEGATIVE	NEG	0.9 % Normal Saline	0.1 mL 0.9% normal saline (NS)

P. berghei NK65 Administration And Animal Tagging

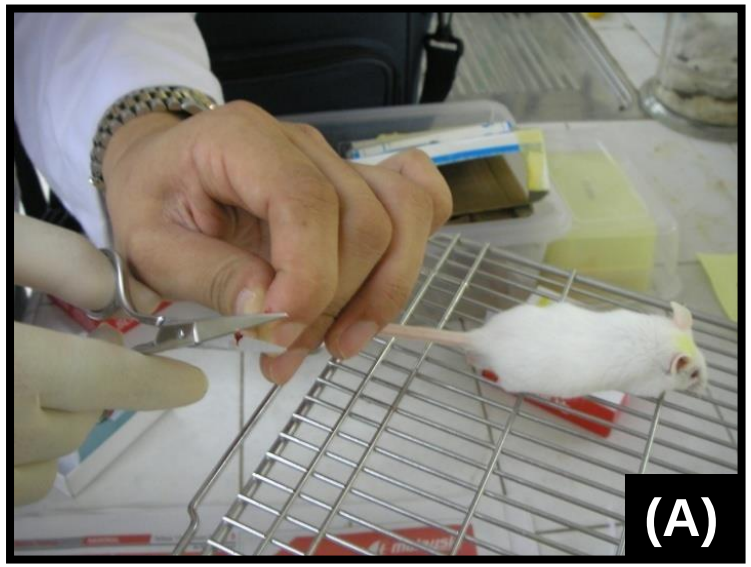


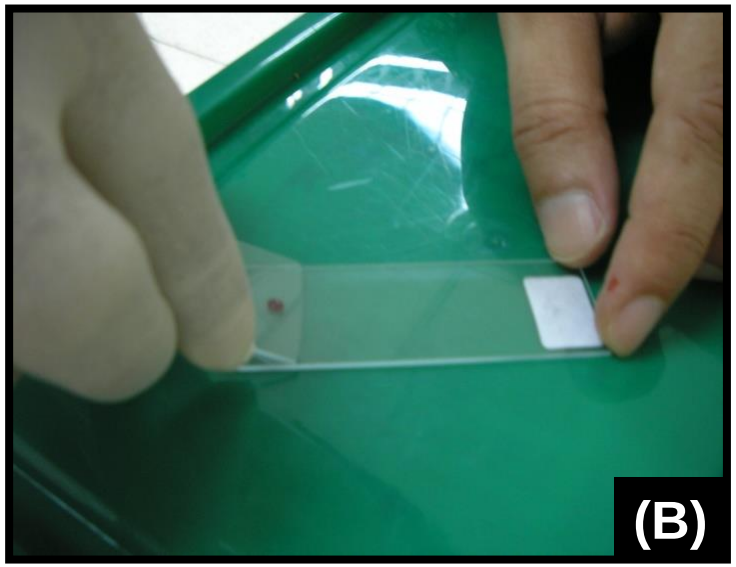


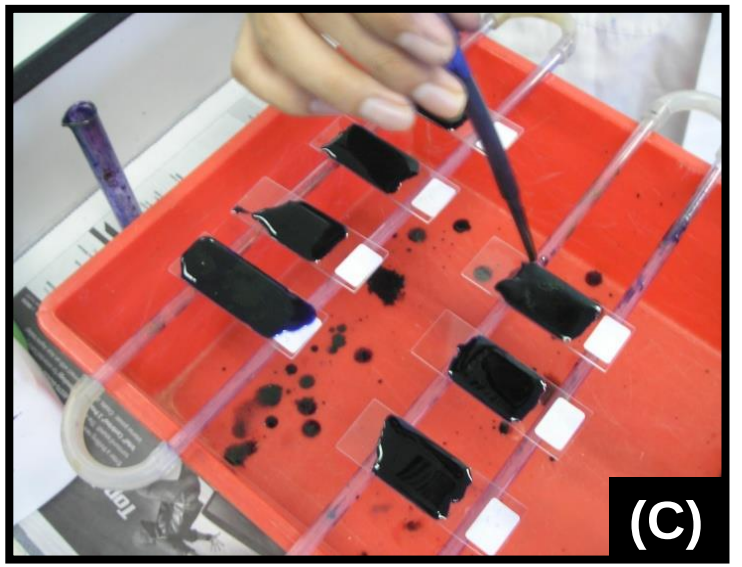


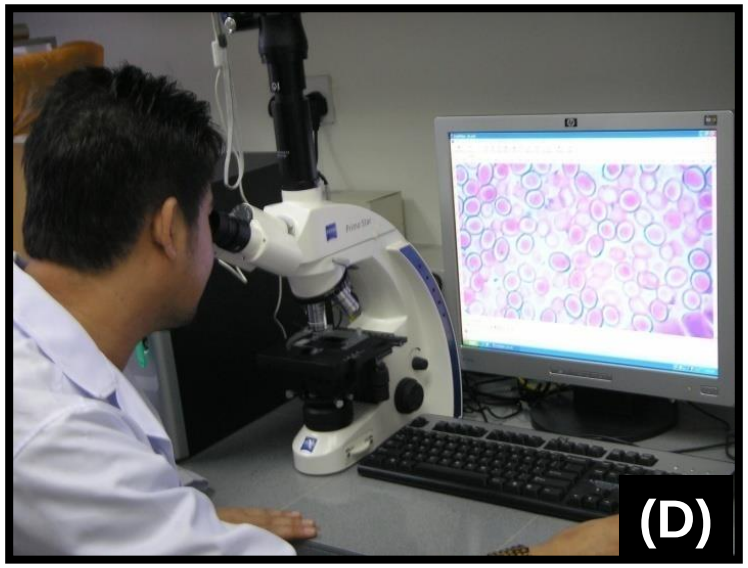


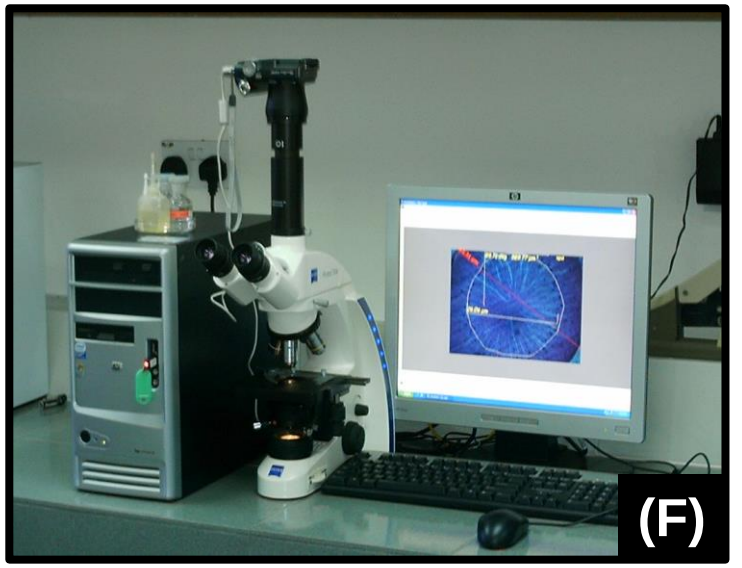
Giemsa Staining And Microscopic Observation











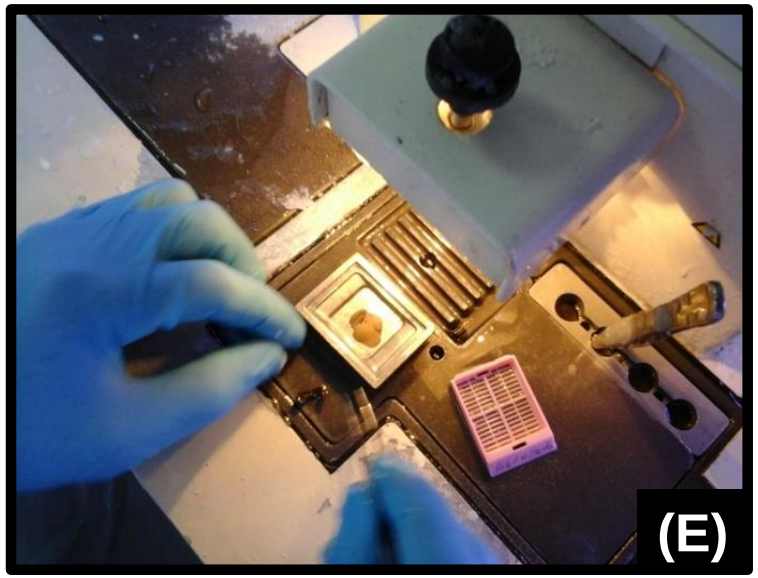
Biochemical Test And Histology Of Liver & Kidney


(A)


(B)


(C)

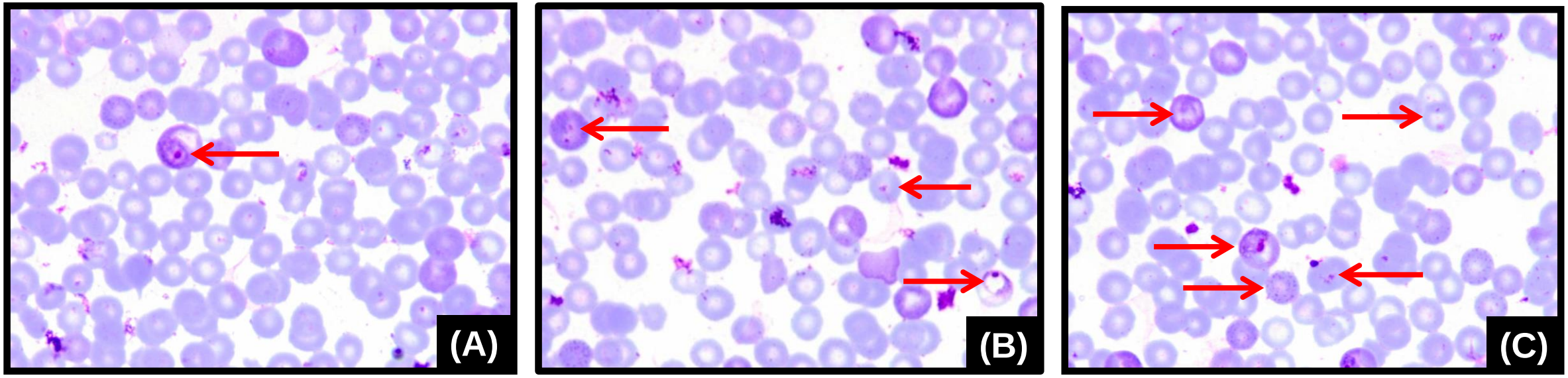

(D)


(E)

(F)

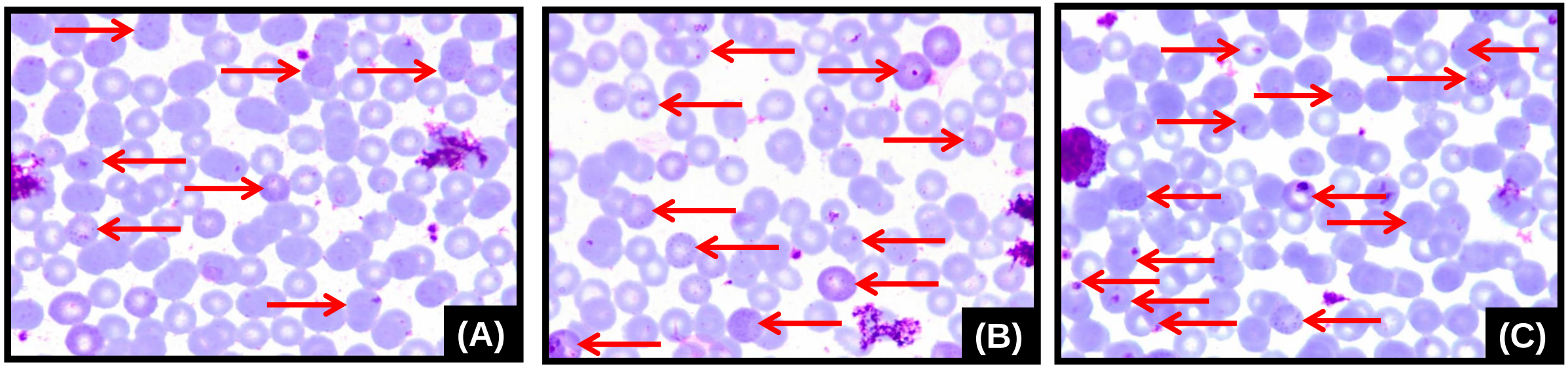
RESULTS & DISCUSSIONS

Thin Blood Film Malaria Parasite (BFMP) Of Preventive Treatment Groups



The thin BFMP of the mice treated with 0.2 mL 100 mg/kg bw sdH_2O-*P. sarmentosum* extract starting from 14 days pre-infection (A), 7 days pre-infection (B) and 3 days pre-infection (C). The slide taken on Day 4 post-infection. The arrows indicated the mice RBC being infected with *Plasmodium berghei* NK65 at all parasite's life cycle stages: immatured trophozoite (ring stage), matured trophozoite, schizont and gametocyte

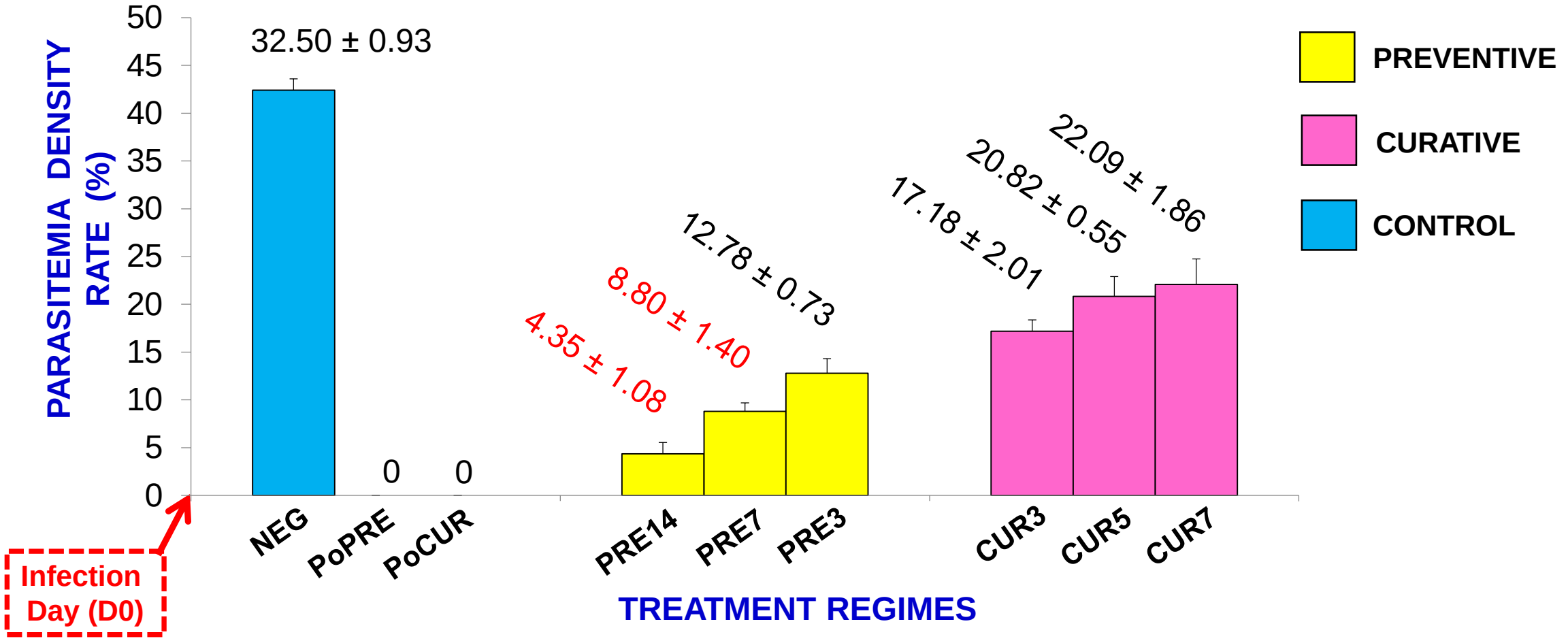
Thin Blood Film Malaria Parasite (BFMP) Of Curative Treatment Groups



The thin BFMP of the mice treated with 0.2 mL 100 mg/kg bw sdH_2O-*P. sarmentosum* extract starting from 3 days post-infection (A), 5 days post-infection (B) and 7 days post-infection (C). The slide taken on Day 4 post-infection. The arrows indicated the mice RBC being infected with *Plasmodium berghei* NK65 at all parasite's life cycle stages: immatured trophozoite (ring stage), matured trophozoite, schizont and gametocyte

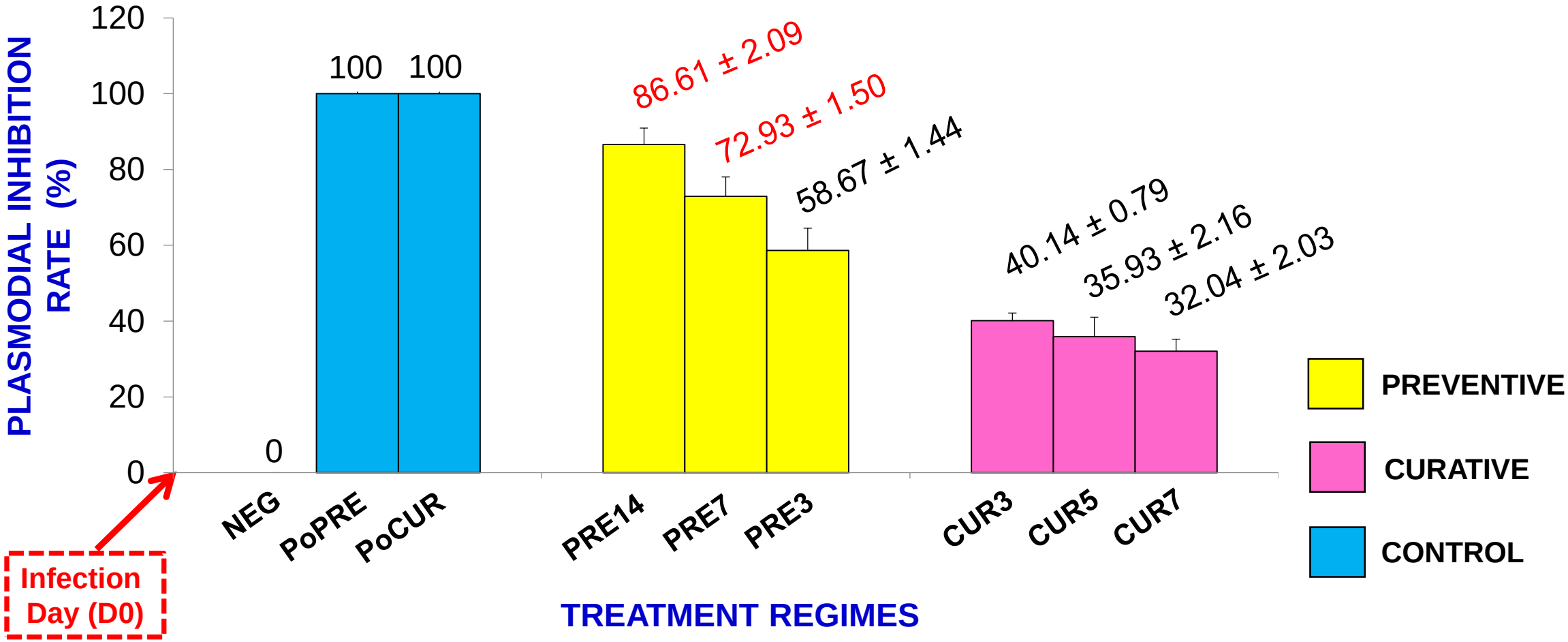
Parasitemia Density Rate (%)

Parasitemia Density Rate (%) of the mice treated with 0.2 mL 100 mg/kg bw sdH_2O-*P. sarmentosum* extract on D4 post-infection for two different regimes groups as compared with four control groups.



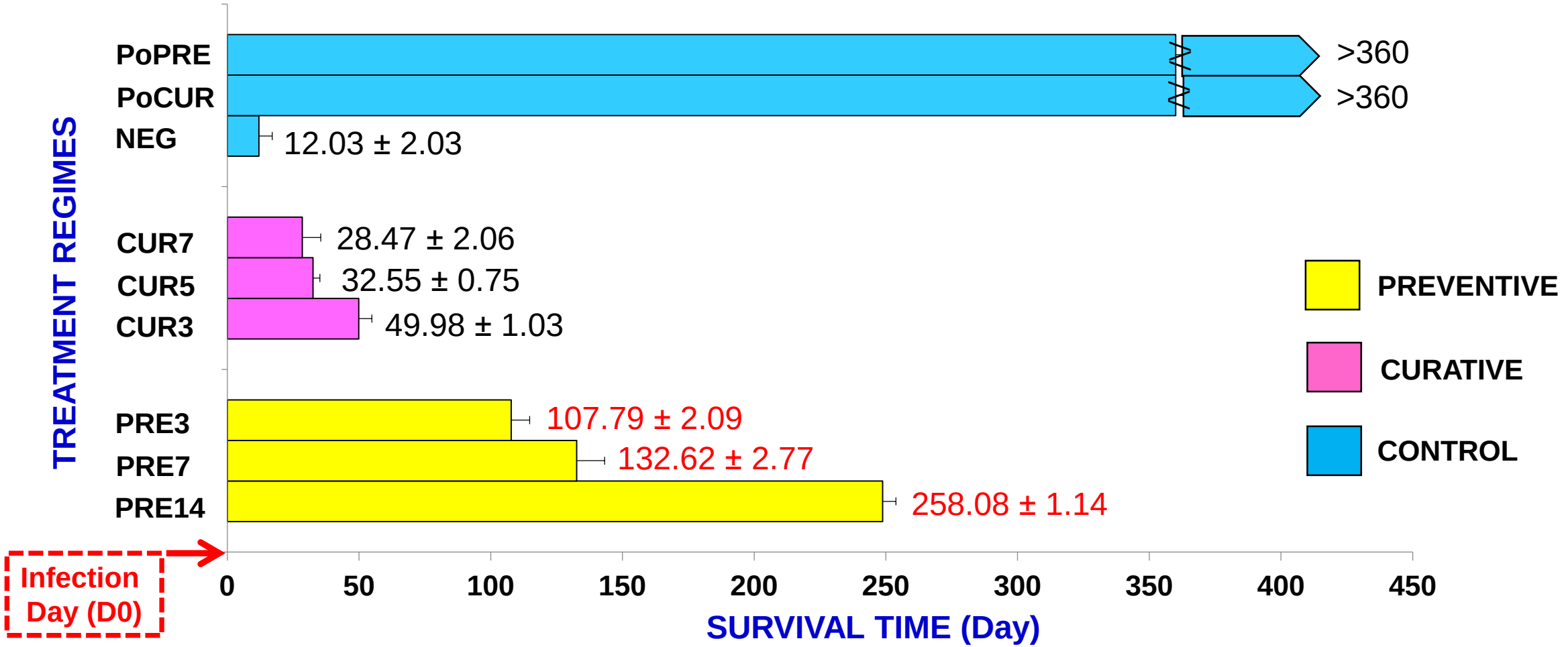
Plasmodial Inhibition Rate (%)

Plasmodial Inhibition Rate (%) of the mice treated with 0.2 mL 100 mg/kg bw sdH_2O-*P. sarmentosum* extract on D4 post-infection for two different regimes groups as compared with four control groups.



Host Survival Time (Day)

Host Survival Time (day) of the mice treated with 0.2 mL 100 mg/kg bw sdH_2O-*P. sarmentosum* extract starting from D0 for two different regimes groups as compared with four control groups.



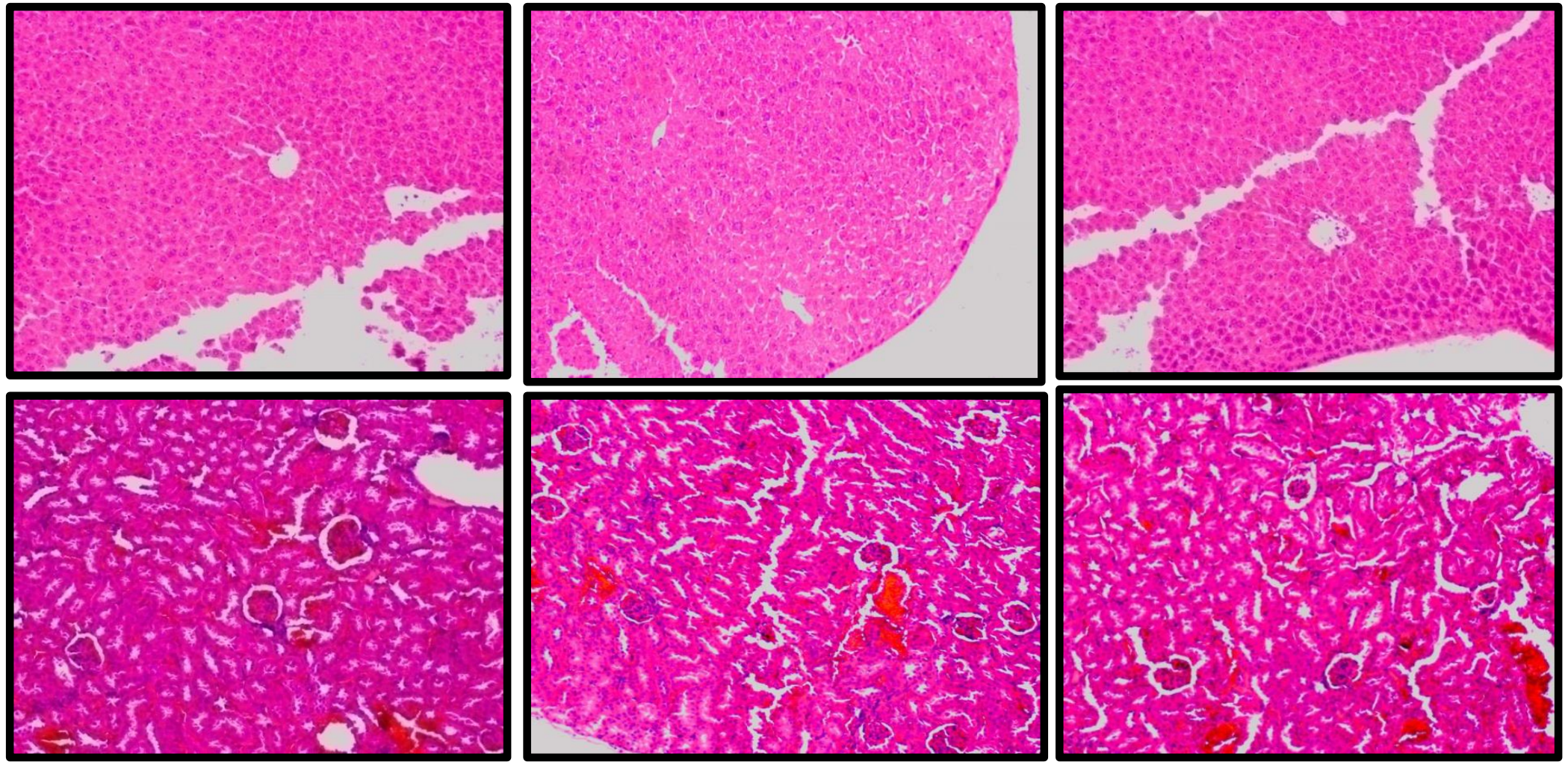
Biochemical Test For Toxicity Assessment

Test	TA	TB	TC	TD	CN	CI	NR	Unit
ALT (*)	41.81 ± 2.14	45.20 ± 1.13	67.57 ± 2.91	90.03 ± 2.02	41.03 ± 3.91	44.83 ± 1.11	**40 – 93**	IU/L
AST (*)	133.13 ± 2.04	125.93 ± 2.12	167.76 ± 2.27	187.01 ± 2.09	111.62 ± 1.19	134.43 ± 4.01	**92 – 206**	IU/L
ALP (*)	62.76 ± 2.33	59.4 ± 2.97	69.2 ± 2.90	68.03 ± 2.10	61.46 ± 2.46	58.32 ± 2.97	**54 – 115**	IU/L
STP (*)	6.12 ± 2.32	7.21 ± 3.81	7.93 ± 2.01	8.83 ± 3.90	6.40 ± 1.01	6.80 ± 3.06	**5.8 – 9.5**	g/dL

TA : Sub-acute regime – Daily treatment (28 days)
TB : Sub-acute regime – Daily treatment (28 days) 2 hours post-infection
TC : Sub-chronic regime – Daily treatment (90 days)
TD : Sub-chronic regime – Daily treatment (90 days) 2 hours post-infection
CN : Control regime – Normal mice without infection and treatment
CI : Control regime – Infected mice on D0
ALT : Alanine aminotransferase
AST : Aspartate transaminase
ALP : Alkaline phosphatase
STP : Serum total protein

(*) All values were expressed as mean ± standard errors (se)
(*) All NR values were referred from Research Animal Resources, University of Minnesota, USA

Organ Histopathology For Toxicity Assessment



CONCLUSIONS

Hypothesis

- 50% of the mice treated with 0.2 mL of 100 mg/kg bw sdH_2O- *P. sarmentosum* extract were survive up to ≥250 days post-infection → third quarter of normal mice life span (Sage et al. 2010; Szenczi et al. 2012).

- No injuries and abnormalities of vital organ and biochemical toxicity effects were discovered on the hosts treated with 0.2 mL of 100 mg/kg bw sdH_2O- *P. sarmentosum* extract at all treatment regimens

- The action of pellitorine ($C_{13}H_{25}ON$) molecule in *P. sarmentosum a*gainst –thiol group of parasite enzymes (*Toxoplasma gondii & Babesia microti*) in which crucial for parasite proliferation (Souza Oliveira *et al*, 2018).

- Bioactive compound of Sarmentamide A in *P. sarmentosum* inhibited the important enzymes (alcohol dehydrogenase, cysteine proteinase and thioredoxin reductase) for the stability of the redox reaction in fungal cells such as *A. fumigatus* & *C. albians* (Tuntiwachwuttikul *et al*., 2006)

Absolute Hypothesis






Absolute Hypothesis

EAT KADUK..!

NO HARM TO EAT AS MUCH AS YOU CAN

RECOMMENDATION

Future Plans

Various solvents of *P. sarmentosum* extract

Mechanism of action

In-vitro antimalarial & toxicity screening

Concentration- & time-dependant alteration

Clinical & molecular approaches

Screening against *P. falciparum* & *P. knowlesi*

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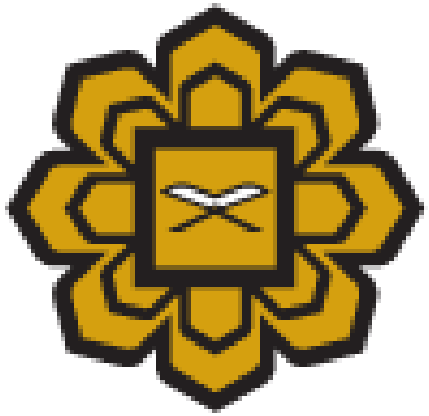
ACKNOWLEDGEMENT



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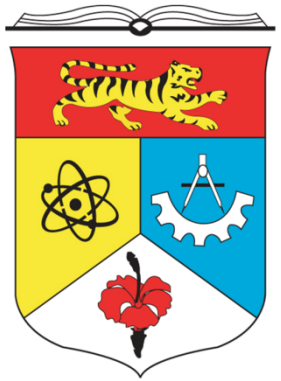

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